Evaluation of the crude phenolic and terpenoid extracts of *Carissa macrocarpa* against *Aphis fabae* Scopoli (Hemiptera: Aphididae) *in-vitro*

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ABSTRACT

Present study was conducted to evaluate different concentrations of crude phenolic and terpenoid extracts of the leaf *Carissa macrocarpa* as alternatives to chemical insecticides in some biological aspects of *Aphis fabae* under *in vitro* condition. The results showed that all plant extract concentrations were effective against adult of *A. fabae* compared with control treatment. The mortality rates were of the adult when used 10 mg/ ml concentration of the crude phenolic and terpenoid extracts after 48 hrs. of exposure 46.4 and 52.3% Compared with 9.2 and 13.8% in control treatment respectively. Also, the crude phenolic and terpenoid extracts have affected reduced the number of birth reached 5.33 and 4.67 nymph/ Female at concentration 10 mg/ mL of the crude phenolic and terpenoid extracts Compared with12.00 and11.67 nymph/ Female in the control treatment respectively.

Keyword: *Carissa macrocarpa*, *Aphis fabae*, Crude phenolic, Crude terpenoid .

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INTRODUCTION

In Iraq, The black bean aphid *Aphis fabae* (Hemiptera: Aphididae) is the main polyphagous pest on agricultural crops. It has been recorded from nearly 120 plant families in the world (Favret and Miller, 2012). *A. fabae* cause large damage to plants by feeding and sucking plant juices leading to the death of plants and reduction in the yield quantitatively and qualitatively, in addition to causing many viral diseases like beet yellows virus (BYV) and brome mosaic virus (BMV) (Stanković *et al.*, 2015). In recently negative effects of chemical insecticides began to appear such as pollution environmental, the appearance of resistance to pesticides by pests and damage to non-target organisms (Bommarco *et al.*, 2011). In order to rectify these problems, scientists in scientific research centres have begun looking for safe alternatives to the environment and effective in the control of the pest. One of these alternatives is plant extracts from diverse botanical families. Many plant species produce substances that protect them by killing or repelling the pests that feed on them because of the natural compounds present in them such as flavonoids, terpenoids, and phenolics (Acheuk *et al.*, 2017). Natural pesticides have many advantages over synthetic ones and may be more cost-effective as a whole, considering the environmental cost of chemical alternatives. Natural pesticides are biodegradable, barely leave residues in the soil (Cooper and Dobson, 2007). In addition, they are cheaper and more accessible in less developed countries. The *Carissa macrocarpa* (Apocynaceae) is characterized chemically by the presence of various secondary metabolites such as flavonoids, saponins, triterpenoids/steroids, anthraquinones, tannins and carbohydrates at different levels in different extracts of different plant organs (Khalil *et al.*, 2015). There are no previous studies on the use of extracts leaves *C. macrocarpa* against the pest. So, the present study was intended to test different concentrations of the crude phenolic and terpenoid extract of leaves *C. macrocarpa* on some aspects of life performance of *A. fabae* in the vitro.

MATERIALS AND METHODS

The study was conducted under laboratory conditions at Department of Field Crops, College of Agriculture, Al Qasim Green University, Iraq-Babil Province during (2017-2018).

Insect collection and rearing

Nymphs and adults of *A. fabae* were collected from the fields of the College of Agriculture,

Al Qasim Green University. The aphids were reared on Petri dishes in an incubator with a temperature 25±2º C and humidity 65±5% as per the method described by Butt *et al*. (1994).

Preparation of Plant Extracts

The *C.macrocarpa* leaves were collected from Hillah city is Iraq in December 2017. The leaves were washed completely with running water and once with sterile refined water, and dried under shade. Phenolic extraction from *C. macrocarpa* leaves was done as per the method described by Ribereau–Gayone (1972). The terpenoid compounds were extracted following the method described by Harborne (1984). The extracts were filtered and the filtrate was evaporated under reduced pressure to obtain the crude. A stock solution of phenolic plant extract was prepared by dissolving 1 g of sticky leaf extract with 3 mL of ethanol solvents. Complete the volume to 100 ml by adding sterile water of a final concentration of 10 mg/mL. The above process was repeated several times to obtain other dilutions 7.5, 5 mg/mL. The control treatment consisted of 97 ml of distilled water and 3 mL of ethanol, while, a stock solution of terpenoid plant extract was prepared by dissolving 1 g of sticky leaf extract with 1.5 mL of ethanol solvents with 1.5 mL of chloroform solvents. Complete the volume to 100 mL by adding sterile water of a final concentration of 10 mg/mL of from this stock solution two different concentrations were prepared 7.5, 5.0 mg/mL. The control treatment consisted of 97 mL of distilled water and 1.5 mL of ethanol solvents with 1.5 mL of chloroform solvents liquid. Paraffin1% and 1-2 drops of tween were added to each concentration as an adhesive agent and surfactant respectively.

Bioassay Test

The effects of crude phenolic with their different concentrations were evaluated against adult of *A. fabae* by taking 10 adults placed on the leaves broad bean was prepared in Petri dishes 9 cm diameter and a piece of wet cotton was placed under it. Four replicates for each concentration were done. These replicates were treated with plant extract concentrations previously mentioned. In quantity it was 1 mL per replicate. The Petri dishes were closed after making holes for ventilation. The dishes were surrounded using adhesive tape to prevent the nymphs and adults from going out of the dishes. Then they were transferred to the incubator with a temperature of 25±2^{O}C and humidity 65±5%. The mortality of nymphs and adults was calculated after 48 hours of treatment. To calculate female productivity 20 first-instar nymphs were taken and treated by the extract and concentration as in the previous case. Growth has been pursued to reach the adult stage. Adults were isolated after it appeared, it was taken from them (4 adults/concentration) and three replicates were maintained.

Data analysis

The data obtained were analyzed using GenStat package 3 (3rd edition) by Completely Randomized Design and Factorial randomized design experiments. The least significant difference L.S.D test was used to compare between means. Data on mortality were corrected according to Abbot (1925). Mortality Percentages data were transferred angular for statistical analysis except female productivity.

RESULTS AND DISCUSSION

The effects of different concentrations of the crude phenolic and terpenoid extracts of the leaf *C. macrocarpa* on the mortality percentage of adults of *A. fabae* are shown in Table 1. The mortality rates were significantly varied with concentrations used in the treatments ($P < 0.05$). A direct correlation between an increase in adults mortality with the increase in the concentration of the crude phenolic and terpenoid extracts was found.

Adult mortality in the crude phenolic extracts ranged between 9.2% in control treatment to 46.4% at a concentration of 10 mg/ ml. while the greatest percentages of mortality of adult 52.3 % with the highest concentrations 10

mg/ml of the crude terpenoid extract plant compared with 13.8% mortality in the treatment controls. In this respect the increase in mortality of adult *A. fabae* may be due to the *C. macrocarpa* extracts were contained on many natural compounds Similar to Vehicles used in insecticide manufacturing such as flavonoids, saponins, triterpenoids, steroids, anthraquinones, tannins (Khalil *et al*.,2015), Which have toxic and deadly effects for treated insects. (Omran, 2009) found that the alcohol extracts of, *Euphorbia peplus* and *Melia azedarich* were highly toxic to the adult, *A. fabae*. Also, she found that the adults' high mortality percentage was reached 58.88% and 39.99 % respectively. These results are in agreement with those of Rathi and Al- Zubaidi (2011) with different extract plant and pests who reported that the crude phenolic extracts of *Nerium oleander* leaves increased the mortality of stages whitefly *Bemisia tabaci*.

Table 1. The effects of leaves crude phenolics and terpenoid extracts of *C. macrocarpa* on the mortality of adult*A. fabae*at 48 hrs.

Extractconcentration mg/ml	Crude phenolics Extracts	Crude terpenoid Extracts	Mean Concentration
0.0	9.2	13.8	11.5
5.0	36.2	40.6	38.4
7.5	42.1	47.9	45.0
10	46.4	52.3	49.4

Table 2 shows that different concentrations of the crude phenolic and terpenoid extracts of the leaf *C. macrocarpa* were significantly affected by the female productivity of adults *A. fabae*. The effect was inverse, where productivity decreased as the concentration increased. The average numbers of births adult female of *A. fabae* significantly reduced from 12.00, 11.67nymph/female in the control treatment to 5.33,4.67nymph / Female respectively, at 10 mg/ml a concentration of crude phenolics and terpenoid extracts of *C. macrocarpa*. Omran (2009) mentioned that the plant extracts had reduced the number of birth reached 3.99 and 5.55 respectively in the *E. peplus* and *E. helioscopia*. Natural pesticides act as growth inhibitors and feeding deterrents as a result of the disturbance of hormonal and other physiological systems like productivity, nutritional requirements, chemoreception, toxicity (Slama, 1969). Jose and Sujatha (2017) reported that terpenoids, coumarin and phenols, present in the methanol extracts of *Gliricidium sepium* exhibited significant antifeedant activity instar larvae of *Helicoverpa armigera*. Similar results were also recorded by different workers in different extract plant and pests (Sharma and Sohal, 2016).

Table 2. Productivity (nymph / Female) of adult *A. fabae* treated with leaves crude phenolics and terpenoid extracts of *C. macrocarpa*

Treatment	concentration mg/ml				Mean Extracts
	0	5	7.5	10	
Crude phenolics extracts	12.00	8.67	6.67	5.33	8.17
Crude terpenoid extracts	11.67	8.00	6.33	4.67	7.67
Meanconcentration	11.83	8.33	6.50	5.00	

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